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ANALYSIS OF WATER QUALITY USING PHYSICOCHEMICAL AND ICROBIOLOGICAL PARAMETERS IN VERJESHWARI RESERVOIR OF MUMBAI, INDIA

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ABSTRACT

A water sample was collected from hot spring of Vergeshwari for microbial and physiochemical study. Water sample is subjected to physiochemical analysis like pH, TDS, Acidity, salinity, dissolved oxygen (DO), biological oxygen demand (BOD) and chemical oxygen demand (COD). The BOD and COD were found in lower amount. Four bacterial strains were isolated from the water sample and all they are spore former, gram positive and gram negative, motile and non motile in nature. Among them one bacterial species was identified to Bacillus cereus and other as Acinetobacter sp. The performed MPN method showed the absence of coliform bacteria in the water sample. The Bacterial isolates were having potential to produce the hydrolytic enzymes such as protease, amylase, lipase, cellulose, invertase were analyzed respectively.

KEYWORDS: Acinetobacter, Amylase, Bacillus, Cellulase, Microbial and Physiochemical Analysis, Protease, Thermophiles

INTRODUCTION

Organisms often live in places where seem to be unliveable. Thermophiles are heat-loving, with an optimum growth temperature of 70°C or more, but these are only approximate. Some hyperthermophiles require a very high temperature 80°C to 105°C for growth (Brock and Freeze, 1969; Hamilton-Brehm *et al.,* 2005).

Extremophiles, particularly thermophiles are getting recognition mainly due to their biotechnological applications. The enzymes isolated from some thermophilic organisms have proven to be of great importance in biotechnology industry due to their ability to function under high temperature conditions. Among the enzymes, microbial esterases (E.C. 3.1.1.1, carboxyl ester hydrolases) and lipases are of substantial interest because of their prospective biotechnological application such as the modification of triglycerides for fat and oil industry, synthesis of flavor esters for food industry, resolution of racemic mixtures used for the synthesis of fine chemicals for the pharmaceutical industry etc. (Molinari *et al*., 1996).

Therefore, the exploration for new microbial enzyme sources is vital for the advancement of new thermostable and organic solvent resistant enzymes and their applications. Thermostable enzymes are usually screened from thermophilic or hyperthermophilic organisms (Gowland *et al*., 1987).

The ability of thermophiles to proliferate at elevated temperatures is attributed to the thermally-stable macromolecules they possess (Zeikus *et al*., 1998). As a consequence of growth at high temperatures and unique macromolecular properties, thermophiles exhibit high metabolic rates, thereby generating greater end-products despite lower growth rates compared to mesophiles. They also provide physically and chemically stable enzymes that are of

significant use to industries (Haki and Rakshit 2003). Thermophiles have provided an interesting and challenging platform for researchers since the time of their discovery. However, due to difficulties in isolation and maintenance of the pure culture, their diversity in thermal habitats remains to be explored (Kikani and Singh 2011).

The present study is an attempt to isolate and characterize thermostable enzyme producing thermophilic bacterial strains from the Vergeshwari hot spring, Mumbai, India.

MATERIALS AND METHODS

Sampling and Isolation of Bacteria

Water samples were collected in aseptic culture tubes at different distances from the source (where the water oozes out) of the Hot springs of Vergeshwwari.

Serial dilution method was used to isolate thermophilic bacteria in 1.0% LB medium supplemented with sterile water of the hot springs (which contain all the naturally occurring salts) for aerobic isolates and YE medium used for anaerobic bacteria according to Clark *et al*. (1958)[8] and Takahata *et al.* (2000).

Temperature of the water was measured on the spot where it oozes out. All other characterization/analysis was made in the laboratories (Allen, 1974).

Chemical Analysis

The water chemistry was determined in triplicate by using standard method (Kodarkar, 2006 and Aneja, 2003).

Microbiological Analysis

Different morphological, physiological and biochemical tests like Gram's staining, colony morphology, motility test, and oxidation fermentation test were studied according to Wiegel and Ljungdahl (1981) and MacFaddin (2000).

SCREENING OF ENZYMATIC ACTIVITIES

Amylases

Starch hydrolysis test was used to screen the bacterial isolates showing amylolytic properties. The starch agar plates were streaked by microbial isolates followed by their incubation at 37°C for 24 hours. After incubation, 1% iodine solution (freshly prepared) was flooded on the starch agar plate. The presence of blue color around the growth are as indicated negative result, and a clear zone of hydrolysis surrounding the growth areas indicated a positive result **(**Hamilton et al., 1999).

Amylase Production

Freshly prepared inoculum was used to inoculate the production medium. To prepare the inoculums, a loopful of bacterial isolate was transferred in 100 mL of the medium containing (g/L) starch 10, peptone 10, yeast extract 20, KH_2PO_4 0.10, $CaCl_2.2H_2O$ 0.10, $MgSO_4.7H_2O$ 0.50 and $FeSO_4.7H_2O$ 0.02. The flask was loaded on a rotary shaker incubator at a speed of 200 rpm at 37°C for 24 hours. Amylase production was carried out by submerged fermentation in Erlenmyer Flask. The flask was loaded on a rotary shaker incubator at a speed of 200 rpm at 37°C for 24 hours. After incubation, fermented broth was centrifuged at 8000 rpm for 20 minutes in a cooling centrifuge. After specific time intervals, samples were taken out to determine the enzyme activity and protein concentration.

Amylase Assay

Amylase was determined by spectrophotometric method as described by Sharma, 2007. One unit is defined as the amount of enzyme required to liberate one µmol of sugar reduction per minute by applying the following formula by Haq *et al*., 2002.

IU/mL/min = (Activity of enzyme × 1000)/ (Molecular weight of Maltose × Incubation time)

Cellulases

The ability of the thermophilic isolates to produce cellulose was tested by placing a loopful of bacteria of each isolate into the centre of a TT agar plate containing 1% (w/v) carboxymethyl cellulose (CMC). Petri plates with this medium were inoculated from 16 h old cultures into the centre of every plate and then incubated for 72 h at 55°C. After the incubation period, plates were flooded with 0.1% (w/v) Congo red solution for 1 to 2 min followed by washing the plate with 1 M NaC1 to detect the presence of clear halos around bacterial colonies that secrete cellulases (Patel et al., 2006).

Cellulase Activity Measurements

Organisms were shaking cultured (150rpm) in liquid broth, supplemented with 0.3% CMC for 12h at 55°C, and used to inoculate the production medium. Cellulase activities were measured for cells grown in cellulolytic medium (100ml) supplemented with CMC in incubator at 150rpm. Aliquots from flask was taken out, centrifuges at 10,000 rpm for 10min and cell free supernatants was used for assay.

Cellulose Enzyme Assay

All enzyme assays was carried out in 50mM sodium citrate buffer (pH 4.8). CMCase activity was determined by Ghose, 1987 with 1% CMC as substrate. Release of reducing sugars in 30min at 50C was measured by DNSA method by Miller, 1959.

Lipases

The ability of the thermophilic isolates to produce lipases was tested, using the method described by Choo *et al*. (1998) with a slight modification, by culturing each isolate on a TT agar plate containing 1% (v/v) tributyrine and 0.01% (w/v) $CaCl_2.2H_2O$. The pH was adjusted to 7.5 with 3 M NaOH. A loopful of each of the different thermophilic isolates was spotted into the centre of each plate and then incubated at 55°C. Plates were followed up for 182 h of incubation to check the formation of the clearing zones around the bacterial colonies that secrete lipases. A positive control *Escherichia coli* was used (incubated at 37°C).

Lipase Enzyme Production

The microbes were grown in basal production medium containing (g L-1): glucose 2.0; K2HPO4 1.0; NH4Cl 5.0; MgSO4.7H2O 0.1; coconut oil 2.0% (v/v) and pH 7.0 for enzyme production (Rathi *et al*., 2001). The cells were allowed to grow in this medium at 30±2 ºC on a rotary shaker, shaking at 150 rpm. Samples were withdrawn at different time interval and centrifuged at 10,000 g for 10 min at 4 ºC to get cell free supernatant. Enzyme activity was determined from the cell free supernatant

Lipase Assay

Lipase activity was determined by the method of Macêdo *et al*., (1997), with the following modifications. Lipase assay was performed with olive oil emulsion, which was prepared by mixing 25 mL of olive oil and 75 mL of 7% gum acacia solution in a homogenizer. The reaction mixture containing 5 mL of olive oil emulsion, 2 mL of 10 mM phosphate citrate buffer (pH 7.0) and 1 mL of the culture broth supernatant was incubated at 30±2 ^{o}C for 30 min in orbital shaker shaking at 150 rpm. The reaction was immediately stopped after incubation, by adding 1 mL of acetone-ethanol mixture (1:1, v/v) and the liberated free fatty acids were titrated with 0.05 N NaOH using phenolphthalein as an indicator. The assay was also performed using a phosphate citrate buffer of pH 5.0 and 6.0. One unit of lipase activity was defined as the amount of the enzyme, which liberated 1 µmol of fatty acid per min (Sharma *et al*., 2009).

Proteases

The culture fluid was spread on screening medium agar plate and the protease producers were detected by plating on gelatin agar, which contained 0.5% gelatin, 0.3% peptone and 1% NaCl. The strains showing clear zones were identified as a protease producer.

Culture and Growth Conditions for Protease Production

For protease production, the isolated bacterium was grown in a medium (pH=8.0) consisting of 0.5 % (w/w) glucose, 0.75% (w/w) peptone, 0.5 % (w/w) $MgSO_4$, 0.5% (w/w) KH_2PO_4 and 0.01% (w/w) $FeSO_4$, in orbital shaking incubator at 60 °C and 140 rpm for 72 h. After incubation, the culture broth was centrifuged at 4 °C and 3600 rpm for 30 min. The cell precipitate was discarded to obtain the supernatant, which was used as crude protease preparation for further purification by Mehta *et al*., 2006.

Assay of Protease Activity

Enzyme activity was measured according to the method described by Kunitz *et al*..1945. The sample contained enzyme solution and 0.5% (w/v) casein in 0.1 M potassium phosphate buffer (pH=8.0) and was incubated at 60 °C for 10 min. This reaction was terminated by the addition of 5% (w/v) trichloroacetic acid (TCA) solution and then centrifuged to remove the resulting precipitate. Protease activity was estimated using a tyrosine standard curve. One unit of alkaline protease activity (U) was taken as the amount of enzyme liberating 1µg of tyrosine/min under the assay conditions.

RESULTS AND DISCUSSIONS

The water sample was collected from hot spring of Vergeshwari in thermal containers. The total bacterial population of the hot spring of Vergeshwari was approximately 20 x 10^2 CFU ml^{-1} water. According to Reda the higher level of temperature affects the qualitative as well as quantitative structure of microbial communities and this was found in several studies that temperature influences microorganisms by adversely affecting their growth, morphology and biochemical activities, resulting in decrease biomass and diversity (Reda, 2007, Brock, 1978 and Ljungdahl, 1979). A total six isolates were randomly selected from the thermus agar plate. All isolates were screened for their ability to grow at different temperature ranging from 20 to 60°C. At 20 and 60°C the growth of the isolate was drastically decreases but at 50°C the most advantageous growth of all the isolate was analyzed. The isolated four samples were biochemically analyzed and showed variable characters shown in Table 1. It was found that among the bacterial isolates Isolate 1 was gram positive, motile, spore forming bacilli and Isolate 2 to 7 was gram negative, nonmotile, spore forming bacilli.

Table 1: Cellular and Biochemical Characteristics of the Isolated Thermophilic Bacteria

Isolate	GS	Shape	TSIA				U	Cat	Oxid	NR	I	MR	VP	Cit	Starch	Isolate identified
			Butt	Slant	Gas	H2S										
1	Pink +ve	Rod Shape	Y	P	-	-	+ve	+ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	Bacillus cereus
2-7	Purple -ve	Rod Shape	Y	P	-	-	-ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve	+ve	Acinetobacter

Physiochemical Analysis: The Table 2 present physiochemical analysis of water sample was collected from hot spring of Vergeshwari.

Sr. No	Test	Result
1	pH	7.9
2	Acidity	275
3	Carbon Dioxide (Co2)	22
4	Disslove Oxygen (Do)	4 Mg/L
5	Chloride (Cl2)	1420 Mg/L
6	Total Dissolve Solid (Tds)	188 mg/l
7	Biological Oxygen Demand (BOD)	32.8mg/l
8	Chemical Oxygen Demand (COD)	8mg/l

The WHO has suggested a limiting value of 500mg/L of TDS for potable water. In the present investigation this limit is not crossed on either side by the sample under study. Such value is acceptable for domestic use. According to Mishra an overwhelming value of TDS may be increases the conductivity values of the water sample (Mishra *et al.*, 2008). Dissolved oxygen present in drinking water adds taste and it is highly fluctuating factor in water. In this study dissolved oxygen content was 32.8 mg l^{-1}.

The maximum allowed value of chemical oxygen demand (COD) is 10 mg/L in drinking water. The present samples have registered 8 mg/L. This value is much lower than the limit. According to WHO such water may be useful for the drinking purpose. Most of the water samples contain significant amount of organic matter that provides nutrition for the growth and multiplication of microorganisms.

The most probable number (MPN) is a suitable and widely used method to determine the microbial quality of water (Kodarkar, 2006). The present investigations have rendered all tests was negative indicates the absence of coliform bacteria. The production of biotechnologically important enzymes was analyzed for protease, amylase, lipase, cellulase and invertase respectively (Table 3). Proteolytic enzymes predominantly proteases, have become an important and fundamental part of the industrial processes including pharmaceuticals, food products and laundry detergents (Rao *et al.*, 1998). The amylase is extensively used in many industries including starch liquification, brewing, food, paper, textile and pharmaceuticals (Ohdan *et al.,* 2000).

Table 3: Enzymatic Activities of Isolates

Isolate	Amylase	Protease	Cellulase	Lipase	Invertase
1	40mcg/ml	490mcg/ml	160mcg/ml	270mcg/ml	60mcg/ml
2	60mcg/ml	1040mcg/ml	340mcg/ml	310mcg/ml	80mcg/ml

CONCLUSIONS

In conclusion, the isolated thermostable *Bacillus cereus and Acinetobacter* sp have potential to produce a significant protease, amylase and cellulose enzymes. The isolated enzymes from the B. cereus may produce an extremozyme like protease and amylase which could tolerate higher temperature as well as hydrolyse proteins and starch. Vergeshwari hot springs have large amount of potential microbial storages and can be used as a source for different thermostable biological products like enzymes. However, more work is clearly needed to elucidate the potential of these enzymes.

REFERENCES

1. Brock, T.D. and Freeze, H. 1969. *Thermus aquaticus* gen. n. and sp. n., a nonsporulating extreme thermophile. J. Bacteriol., 98(1): 289-297.
2. Hamilton-Brehm, S.D., Schut, G.J. and Adams, M.W. 2005. Metabolic and evolutionary relationships among *Pyrococcus Species*: genetic exchange within a hydrothermal vent environment. J. Bacteriol., 187(21):7492-7299.
3. Molinari F., Brenna O., Valenti M. 1996. Isolation of a novel carboxylesterase from *Bacillus coagulans* with high enantioselectivity toward racemic esters of 1,2-O isopropylideneglycerol. Enzyme. Microb. Tech. 19:551-556.
4. Gowland, R.K., Kernick, M. and Sundaram, T.K. 1987. Thermophilic bacterial isolates producing lipase. FEMS Microbiol. Lett. 48:339-343.
5. Zeikus, .J, Vieille, C. and Savchenko, A.1998. Thermozymes: biotechnology and structure-function relationships. *Extremophiles*, 2(3): 179-183.
6. Haki, G. and Rakshi, S.2003.Developments in industrially important thermostable enzymes: a review. Bioresour Technol., 89(1):17-34.
7. Kikani, B. and Singh, S.2011. Single step purification and characterization of a thermostable and calcium independent α-amylase from Bacillus amyloliquefaciens TSWK1-1 isolated from Tulsi Shyam hot spring reservoir, Gujarat (India). Int J Biol Macromol., 48(4):676-681.
8. Clark, D.S., Bordner, P., Geldrich, E.F., Kabler, P.W. and Huff, C.B.1958. Appl. Microbiol. International Book Company, New York, 27-53.
9. Takahata, Y., Nishijima, M., Hoaki, T. and Maruyama, T. 2000. Distribution and physiological characterization of hyperthermophiles in the Kubiki oil reservoir in Niigata, Japan. Appl. Environ. Microbiol., 66: 73-79.
10. Allen, S.E.1974. Chemical analysis of ecological materials. John Wiley and Sons, New York, USA.
11. Wiegel, J. and Ljungdahl, L.G. 1981. *Thermoanaerobacter ethanolicus* gen. nov., sp. nov., a new extreme thermophilic, anaerobic bacterium. Arch. Microbiol., 128: 343-348.
12. Famacddin, J.F. 2000. Biochemical tests for identification of medical bacteria. (3rd Ed). Lippincott Williams & Wilkins.
13. Hamilton, L.M., Kelly, C.T., Fogarty, W.M.1999. Production and properties of the raw starch-digesting α-amylase of; Bacillus; sp. IMD 435. Process Biochem.,35(1):27-31.

14. Sharma, K. 2007. Manual of Microbiology Tools and Techniques. 1. Delhi India: Ane Books., p. 141-71.
15. Haq, I., Abdullah, R., Ashraf, H., Athar Shah, H.2002. Isolation and screening of fungi for the biosynthesis of alpha amylase. Biotechnol, 2:61-6.
16. Patel, M.A., Mark, S., Ou, Roberta, H., Hemry, C.A., Marian, L.B., Lonnie, O.I. and Shanmugam, K.T.2006. Isolation and characterization of acid tolerant, thermophillic bacteria for effective fermentation of biomass derived sugars to lactic acid, Appl Environ Microbiol., 72: 3228-3235.
17. Ghose, T.K.1987. Measurement of cellulase activities. Pure Appl Chem., 59:257-268.
18. Miller, G.L.1959. Use of DNS reagent for determination of reducing sugar, Analytical Chem., 31: 426-428.
19. Choo, D.W., Kurihara, T., Soda, K., Esaki, N.A. 1998. Cold-adapated lipase of an Alaskan psychrotroph, Pseudomonas sp.strainB11: Gene cloning and enzyme purification characterization. Appl Environ. Micro, 64(2): 486-491.
20. Rathi, P., Saxena, R.K. and Gupta, R. 2001. A novel alkaline lipase from *Burkholderia cepacia* for detergent formulation. Process Biochem, 37: 187-192.
21. Macedo, G.A., Park, Y.K. and Pastore, G.M. 1997. Partial purification and characterization of an extracellular lipase from a newly isolated strain of *Geotrichum* sp. Rev. Microbiol., 28: 90-95.
22. Sharma, A., Bardhan, D. and Patel, R. 2009. Optimization of physical parameters for lipase production from *Arthrobacter* sp. BGCC#490. Ind. J. Biochem. Biophys, 46: 178-183.
23. Mehta, V.J. Thumar, J.T. and Singh, S.P.2006. Production of alkaline protease from an alkaliphilic actinomycete. Biores Technol., 97: 1650-1654.
24. Kunitz, M. 1945.Crystalline soybean trypsin inhibitor. J Gen Physiol., 30: 291- 310.
25. Reda, A.I. Abou-Shanab.2007. Characterization and 16S rDNA Identification of Thermo-tolerant Bacteria Isolated from Hot Springs Journal of Applied Sciences Research., 3: 994-1000.
26. Brock, T.D. 1978. Thermophilic microorganisms and life at high temperatures. Springer-Verlag, New York.
27. Ljungdahl, L.G. 1979. Physiology of thermophilic bacteria. Advanced Microbial Physiology, 19: 149-243.
28. Mishra Arunabh and Bhatt Vasishta. 2008. Physico-Chemical and Microbiological Analysis of Under Ground Water in V.V Nagar and Nearby Places of Anand District, Gujarat, India. E- J. Of Chemistry., 5(3): 487-492
29. Kodarkar, M.S. 2006. Methodology for water analysis, Indian association of aquatic Biologists (IAAB). Pub : 2.
30. Rao Mala, B., Tanksale Aparna, M., Ghatge Mohini, S. and Deshpande Vasanti, V. 1998. Molecular and biotechnological aspects of microbial proteases. Microbiology and molecular biology reviews., 597–635.
31. Ohdan, K., Kuriki, T., Takata, H., Kaneko, H. and Okada, S. 2000. Introduction of raw starch–binding domains into Bacillus subtilis alpha–amylase by fusion with the starch–binding domain of Bacillus cyclomaltodextrin glucanotramferase. Appl. Environ. Microbiol, 66: 3058–64.